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A COMPARISON OF ANTIGENS FOR BACTERIAL COMPLEMENT FIXATION *

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Bacterial complement fixation is one of the methods used at present in testing for immune bodies in serum. The success of the test seems to depend largely on the use of a reliable bacterial antigen. It has been our custom to use a simple antigen, merely a heated washed suspension of bacteria in normal salt solution. Since other antigens have been described by other writers as being preferable, we decided to compare, in a few bacterial complement-fixation tests, this antigen with antigens prepared by other methods, as to specificity, anticomplementary and antigenic properties, and antigenic range. The following antigens were used for the tests:

Antigen 1.—This was an autolysate antigen, originally used by Blake[1] for precipitin tests. Washed bacteria were dried in vacuo and suspended in normal salt solution in the proportion of 10 mg. per c c. This suspension was repeatedly frozen and thawed until a faintly opalescent fluid, free from bacterial bodies, resulted. Dilutions of this opalescent fluid in normal salt were used as antigen.

Antigen 2.—Antigen 2 was prepared according to the method used by Kinsella and Swift.[2] Washed sediment from 24-hour cultures was suspended in 5 c c each of normal salt solution and of absolute alcohol. The precipitate formed was centrifugalized, desiccated in vacuo, ground and weighed. To 10 mg. of the resulting precipitate, 5 c c of 2% antiformin was added, and the suspension placed in the water bath at 56 C. until the bacteria were dissolved. The solution was neutralized with 0.1 N sulphuric acid (litmus indicator), and 1 to 2 drops of 5% sodium thiosulphate were added to free the chlorin, the end results being tested with potassium iodid starch paper. The solution was made up to 10 c c with carbolized normal salt solution and centrifugalized. One c c of this antigen equaled 1 mg. of the dried ground precipitate.

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[1] Jour. Exper. Med., 1917, 26, p. 67.

[2] Jour. Exper. Med., 1917, 25, p. 877.

Antigen 3.—The third antigen was prepared according to a method described by Krumwiede and Noble,[3] a method especially favored by them as a rapid means for the production of a good precipitin antigen. Sufficient antiformin was added to a heavy suspension of bacteria in distilled water, to make a final concentration of 5%. This suspension was boiled for several minutes for the purpose of dissolving the bacteria. When they were dissolved, there was an appearance of translucency. If this appearance was not obtained, the process was repeated until the bacteria were in solution. The solution was neutralized with N/1 hydrochloric acid. Several volumes of 95% alcohol were added, the suspension was centrifugalized, and the supernatant fluid was decanted and used as antigen.

Antigen 4.—Warden [4] considered that specific fats rather than proteins might be the antigenic factor in bacterial complement-fixation antigens and prepared some with that in view, which proved successful. His method of extracting fats was not used by us, but Noguchi's [5] method of extracting a lipoid constituent. Bacterial cultures were washed off with normal salt solution, centrifugalized, and the bacterial sediment dried in vacuo. The dried bacteria were extracted in absolute alcohol. The alcoholic extract was evaporated to dryness, dissolved in ether, and precipitated by acetone. The precipitate was weighed, and 0.3 gm. was dissolved in 1 c c of ether and 9 c c of methyl alcohol. This solution was the stock antigen. For the complement-fixation tests one part of the stock antigen was added to 9 parts of normal salt solution.

Antigen 5.—Antigen 5 was prepared according to the method employed by Olitsky and Bernstein.[6] Bacteria were suspended in distilled water, and the suspension was allowed to autolyze at 60 C. for 1 hour and then at 37 C. for 24 hours. The suspension was then centrifugalized until a clear supernatant fluid resulted. This fluid was normalized with 0.9% salt solution and used as antigen.

Antigen 6.—In the preparattion of antigen 6, Gay's modification of Besredka's method was employed.[7] Cultures grown on solid medium were washed off with normal salt solution. The salt suspension was precipitated with an equal quantity of absolute alcohol, and the mixture centrifugalized. The sediment was dried in vacuo, weighed, and ground into a fine powder with sufficient crystals of salt to make a 2% suspension of dried material in an isotonic salt solution, when a proper amount of water was added. This suspension, not filtered or centrifugalized, was diluted with normal salt solution and constituted the antigen.

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[3] Jour. Immunology, 1918, 3, p. 1.

[4] Jour. Infect. Dis., 1918, 22, p. 133.

[5] Serum Diagnosis of Syphilis, p. 79.

[6] Jour. Infect. Dis., 1916, 19, p. 253.

[7] Kolmer, Infection, Immunity, and Specific Therapy, p. 474.

Antigen 7.—Antigen 7 was an autolysate prepared by a method given by Kolmer.[7] Cultures grown on solid medium were washed off with normal salt solution. The emulsion was centrifugalized thoroughly, and the resulting sediment dried over sulphuric acid. The dried material was ground with crystals of salt, and sufficient distilled water was added to render the solution isotonic. Each c c of the suspension contained about 0.05 gm. of the dried material. The emulsion was then shaken for 24 hours and centrifugalized, and the supernatant (preserved with 0.5% alcohol) used as antigen.

Antigen 8.—Antigen 8 was the heated bacterial suspension in normal salt, mentioned previously as our usual bacterial antigen. Bacteria were washed 3 times in normal salt solution, suspended in normal salt solution, and heated at 56 C. for 1 hour. Heating was for the purpose of killing, not autolyzing, the bacteria.

Although it is generally accepted that the bacterial antigenic property lies either in the bacterial body itself or in the endotoxic substances that are liberated when the bacterial cells are autolyzed, nevertheless entire broth cultures of the 4 bacteria used in the tests were used as antigen and also supernatant (decanted after 2 hours' centrifugalization at high speed) from these cultures. An uninoculated broth was used as a control. The latter had no antigenic property. The supernatant and entire cultures had a wide antigenic range and excellent antigenic properities but both were nonspecific, giving about as strong fixations with other immune serums and even normal serums as with the homologous serums. Both were, therefore, discarded as antigens.

The 8 antigens were made for each of the following bacteria: pneumococcus, streptococcus, B. typhosus and meningococcus.

Rabbits were immunized against the same bacterial strains that were used for the antigens. Each antigen was tested for its anticomplementary unit, for its antigenic unit with the homologous immune serum, for its specificity, and for the antigenic range.

The complement-fixation tests were made by the method previously described by us.[8] The proportions were those used in the original Wassermann test, in $\frac{1}{10}$ quantities. The first incubation period was 1 hour at 37 C., and the second incubation period about 30 minutes at 37 C.

RESULTS

Antigen 1: Blake's autolysate, obtained by repeated freezing and thawing, was anticomplementary in the following amounts: pneumococcus antigen, 0.2 c c; streptococcus antigen 0.5 c c of a 1:10 dilution; typhoid antigen, 0.5 c c; meningococcus antigen 0.4 c c. It was strongly antigenic (table) for antistreptococcus and antimeningococcus serums, but only weakly binding for antipneumococcus and antityphoid serums. The antigenic range for the latter 2 antigens was therefore rather limited. Control serums were slightly fixed with the antistreptococcus and antityphoid antigens. Judged by the results of these tests, the meningococus and streptococcus antigens prepared by this method were excellent, but the pneumococcus and typhoid antigens poor.

Antigen 2: The autolysate produced by antiformin were anticomplementary in the following amounts: pneumococcus, 0.5 c c; streptococcus, 0.05 c c of 1:100 dilution; typhoid, 0.05 c c of 1:1,000 dilution, and meningococcus, 0.05 c c of undiluted antigen. There was no antigenic unit for the hemologous antipneumococcus serum (table), a very low antigenic unit for antityphoid and antimeningococcus serums, and a high antigenic unit (1/1024 of the anticomplementary unit) and consequently a wide antigenic range, for the antistreptococcus serum. This method was considered to produce a good antigen for streptococcus only and was not entirely satisfactory here, since it gave slight fixation of complement with a heterologous serum.

Antigen 3, prepared by the rapid antiformin method, was anticomplementary in the following amounts; pneumococcus, 0.05 c c; streptococcus, 0.05 c c; typhoid, 0.05 c c; meningococcus, 0.2 c c. Its antigenic property was good, and the antigenic range was wide. It gave nonspecific fixation in $\frac{1}{4}$ of the anticomplementary unit in the case of the streptococcus antigen, but the antigenic range here was so great that it was still considered a satisfactory antigen.

Antigen 4, the lipoid antigen, was anticomplementary for pneumococcus in 0.4 c c, for streptococcus in 0.05 c c, for typhoid in 0.1 c c, and for meningococcus in 0.05 c c amounts. The antigenic unit was good in the case of the pneumococcus and streptococcus antigens, being 1/512 of the anticomplementary unit, while in the case of the typhoid and meningococcus antigens it was only 1/8 of the anticomplementary unit and consequently a weak antigen. There was also nonspecific fixation with the typhoid antigen which, together with the low antigenic unit and slight antigenic range, would disqualify it as an antigen for typhoid complement-fixation tests.

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[8] Howell and Anderson, Jour. Infect. Dis., 1919, 25, p. 1.

Antigen 5, autolyzed by heat, was anticomplementary in the following amounts: pneumococcus, 0.4 c c; streptococcus, typhoid and meningococcus, 0.05 c c. Its binding unit was good in the case of pneumococcus and streptococcus and fair in the case of typhoid and meningococcus. The antigens, however, were rather nonspecific in action and on this account not entirely satisfactory.

TABLE 1

COMPLEMENT FIXATION WITH DIFFERENT ANTIGENS

Serums	Antigens							
	1	2	3	4	5	6	7	8
Antipneumococcus	8	0	32	512	512	64	16	16
Normal control	0	0	0	0	8	0	0	2
Antimeningococcus control	0	0	0	0	0	0	0	0
Antistreptococcus	2068	1024	2068	512	256	32	512	4136
Normal control	2	0	4	0	4	4	0	0
Antityphoid control	0	2	4	0	4	4	0	0
Antityphoid	8	2	16	8	16	2	64	64
Normal control	2	0	0	2	2	0	2	4
Antistreptococcus control	2	0	0	2	0	0	2	4
Antimeningococcus	512	16	32	8	16	32	16	128
Normal control	0	0	0	0	0	0	0	0

Numerals = fraction of anticomplementary unit of antigen that gave complete inhibition of hemolysis, i. e., 8 = 1/8 of anticomplementary unit.

Antigen 6, the alcoholic precipitate suspended in normal salt solution, was anticomplementary in the following quantities: pneumococcus, 0.5 c c; streptococcus, 0.1 c c of 1:100 dilution; typhoid, 0.1 c c; meningococcus, 0.05 c c. The antigenic unit was 1/64 of the anticomplementary unit for pneumococcus and 1/32 of the anticomplementary unit for streptococcus and meningococcus. There was practically no binding power for typhoid, 1/2 of the anticomplementary unit. The streptococcus antigen was nonspecific in its action.

Antigen 7, the filtrate from organisms dried, ground, and shaken for 24 hours, was anticomplementary in the following amounts: pneumococcus, 0.05 c c; streptococcus, 0.1 c c; typhoid, 0.1 c c; meningococcus, 0.05 c c. The antigenic unit of the pneumococcus and meningococcus antigens was 1/16 of the anticomplementary unit, of the streptococcus antigen 1/512, and of the typhoid antigen 1/64. There was slight nonspecificity of action with the typhoid antigen only.

Antigen 8, the bacterial suspension, was anticomplementary in the following amounts: pneumococcus, 0.4 c c; streptococcus, 0.05 c c; typhoid, 0.1 c c of 1 : 10 dilution; meningococcus, 0.4 c.c The antigenic units were: pneumococcus, 1/16 of the anticomplementary unit; streptococcus, 1/4136 of the anticomplementary unit; typhoid, 1/64 of the anticomplementary unit; meningococcus, 1/128 of the anticomplementary unit. Pneumococcus was nonspecific in 1/2 of the anticomplementary unit and typhoid in 1/4 of the anticomplementary unit, which lessened the antigenic range for these antigens.

Since these antigen tests were completed, an article has appeared by Matsuwoto[9] on a comparison of the methods of preparing B. typhosus antigen. In the 9 antigens that he used, he found a 14-day broth culture, killed by heating at 60 C. for one hour, to be most antigenic. The antigen tested by us that corresponded most nearly to this was the entire broth culture discarded in spite of its high antigenic quality because of its nonspecificity. The antigen second in antigenic value was a suspension of living bacteria in normal salt solution. This corresponded closely to our suspension of heated bacteria in normal salt solution, which was found to be the most satisfactory antigen for our typhoid bacterial complement-fixation tests.

SUMMARY

In summing up the antigens (table), it appears that the bacterial suspension was at least as satisfactory as any other one antigen. It was not anticomplementary in small quantities; the antigenic unit was better than that of the other antigens for streptococus and typhoid; and it ranked second for meningococcus and ranked fourth for pneumococcus. The antigenic range was good, since even in the pneumococcus test there was complete fixation in 3 dilutions. It, like the other antigens, in low dilutions of the anticomplementary unit, gave nonspecific reactions with certain serums, but the antigenic range was wide enough so that there was marked difference between these reactions and fixation with homologous immune serum. Antigen 1 was the most satisfactory of the antigen preparations for the meningococcus test, and antigens 4 and 5 for the pneumococcus test. Since there is such diversity in the choice of the most satisfactory bacterial antigens for these 4 bacteria, and since the bacterial antigenic factor has not been determined, it would seem advisable before starting an extended bacterial complement-fixation study, as a preliminary, to determine the antigen best suited for the bacteria used in that test.

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[9] Jour. of Immunology, 1920, 5, p. 111.